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SHRINKAGE OF CEMENT-CELLS THE CAUSE OF LEAKAGE AND CREEPING IN GLYCERIN MOUNTS.

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A chemist, thorougly acquainted with the physical and chemical properties of all the known substances in nature, is approached by a microscopist who demands of him a perfect mounting medium for delicate organic tissues. This medium, says the microscopist, must have the following characteristics:

It must be transparent and colorless;

It must possess a high refractive index;

It must mingle readily or dissolve in water or alcohol, or both, and the mixture or solution must be limpid and colorless;

It must be neutral in reaction, and must not affect vegetable colors;

It must not dissolve the resins;

It must be antiseptic;

It must be stable under all ordinary circumstances, not decomposing by exposure to moderate heat or light;

It must be non-volatile in summer's heat, and must not congeal with winter's cold.

In addition to these qualities it should be cheap and easily procurable.

The chemist immediately replies, "I know of one substance, and but one in nature that fulfills all these requirements. Here, my friend, you have it. This substance is propenyl alcohol. It is, as you see, limpid and in thin layers without a trace of color. Even in this bottle it has but a faint tinge of yellow or green, part of which is due to the glass. Its refractive index is 1.475. It mingles in all proportions with water and with alcohol, absolute or dilute, and makes a colorless mixture or solution. It is without effect on litmus or turmeric paper, and does not dissolve the resins (such as damar, balsam, etc.). It is antiseptic in a high degree. It is practically non-volatile and unchangeable under ordinary circumstances, boiling only at 554° F. and crystalizing only when agitated and at very low temperatures. It is cheap and obtainable everywhere."

"Give me," exclaims the microscopist, "give me this inestimable treasure! Propenyl alcohol—the very thing I want!"

Whereupon the chemist hands him a bottle labelled "*Glycerin, C. P.*"

The microscopist's jaw drops. "That stuff! not if I know it. *I*'m not going to have a "certain proportion" of my slides ruined. Don't you know that it is an invention and device of Satan himself, contrived especially for the vexation and ruin of the truly scientific microscopist? Don't you know that it has been tried time and again, by our most eminent microscopical editors and professors, and that they have pronounced it the most perverse and willfully deceiving substance in nature? Don't you know that only about one in a dozen slides made with it is permanent? No sir! If you have nothing better than *that* to offer I'll stick to Canada balsam. Propenyl alcohol indeed!"

This imaginary conversation represents the exact status of glycerine in the microscopical technique of to-day. Here we have a mounting medium almost ideal in its combinanation of those virtues which should recommend it to the microscopist, refused, condemned, almost banished from the work table, on account of its possession, or rather its alleged possession of one grievous fault, viz:

A tendency to leak or creep out of the cells in which it has been cemented.

That this tendency *does* exist, and that it has been the cause of the destruction of innumerable valuable preparations, and consequently of a vast number of disappointments, I am not prepared to deny. In the earlier days of my microscopical work I too was a sufferer from this "tendency"; but I *do* deny most seriously and emphatically that it is due to any inherent vice or quality in the medium itself, which unfits it for work which is intended to be lasting and stable.

Having thus suffered from the peculiar behavior of glycerine above alluded to, and having at the same time in my cabinet many slides which had remained perfect for so many years that I was justified in calling them permanent, I became convinced that the failures were not due to the use of glycerine. I reasoned that if one single preparation out of a thousand remained permanently good, it was proof positive that with the same materials, manipulated in the same manner, and with the same precautions, all would have been equally so. The laws of Nature are invariable and immutable. Given a certain cause and under the same circumstances, an identical result will always be obtained. With this law in my mind I determined to investigate the causes which led to the varying results which were obtained when identically the same materials were used under apparently the same circumstances.

It is unnecessary to enter upon the details of this envestigation, or to report the experiments which finally led to the solution of the problem. It suffices to say that I proved to my satisfaction, as I hope soon to show to *yours*, that the secret lay entirely in *the length of time which was allowed to elapse between the ringing of a slip and the filling and closing the same.* In other words success in making a permanent mount depends entirely (supposing your materials to be what they should) upon the thorough seasoning of the cell-wall.

The reasons for this are so obvious now that the marvel is that I, or some other worker, had not sooner discovered, or rather reasoned out, the source of error.

All cements used in microscopical technology, with the exception of the ordinary gold-size, consist of a gum or resin, hard and solid at ordinary temperatures, dissolved in a more or less volatile fluid, upon the evaporation of which they regain their solidity. The exception, gold-size, hardens and becomes solid partly, but to a very small degree, by evaporation, but mainly by a process of oxidation. The amount of volatilizable fluid used in these cements depends upon the nature of the gum. In zinc-oxide or white-zinc cement, which consists of gum damar, oxide of zinc and benzol, the latter, in the best quality of cement, exists in a quantity nearly, if not quite, equal in volume to both the other ingredients put together, and hence in its evaporation the volume of the cement is reduced nearly, if not quite, one half.

In other words the cell-wall built with it, in the process of hardening, shrinks to only one half its original volume. This shrinkage, owing to the tenacity with which the damar clings to the surface of the glass, is mainly in one direction, i.e., in its vertical proportions, and consequently its containing power is reduced fully to that extent.

Glycerine is practicably a non-compressible and non-volatile fluid, and it is self evident that if a cell be filled therewith before the walls, by the evaporation of their volatile constituent, have reached a point where further shrinkage will not occur, something must give way. This break will occur at the weakest point of the structure, usually the cover-glass or the line of juncture between it and the cell-wall.

This is plain to everyone. When an irresistible force meets an unyielding body a crash is inevitable. The catastrophy may follow very quickly after the filling of the cell, or it may be many weeks or months, or possibly years in coming, but come it must. The length of delay is dependent almost solely upon the degree of seasoning which the cell-walls had enjoyed before being filled and closed.

HOW LONG SHOULD A CELL-WALL BE SEASONED BEFORE IT IS SAFE TO USE IT?

In view of what has been said this becomes a vital question as regards the slide, and a very important one to those who prefer glycerine as a mounting medium, and in order to settle it, for the benefit of myself and others, I instituted a series of experiments which I will briefly describe. I soon found that the external appearances, the looks of the cement and its resistance to the pressure of the nail, did not constitute a safe criterion, especially in large and deep cells. The surface of these might be of glassy hardness, but when they were cut into the interior would be found of almost putty-like consistency. I therefore devised, from the debris of an old aneroid barometer and some other portions of apparatus at hand, an instrument for measuring the amount of shrinkage which a cell of given dimensions would undergo within given periods, and under known conditions as to temperature, etc. The construction of this instrument is as follows:

The base consists of a T shaped bed-plate of brass, each arm of which is about 4.5″ long, 1.5″ wide, and 0.3″ thick. It stands on three legs. The transverse arm carries the slide, rigidly secured by screws arranged at each end. Two levers are so geared that the vertical movement of a rod is shown, multiplied one hundred times on the scale. These levers are perfectly balanced by wrapping fine wire on the shorter arms. Standards curved so as to economize space, are so arranged that by means of two screws they may be moved up or down within minute limits, thus enabling me to bring the index to the zero of the scale without interfering with the gearing, at any stage of a test. The bearings of the fulcral pivots, are knife edged. The scale is of ivory, originally divided into fiftieths of an inch, but subdivided by me, by the eye alone, into one hundred and fifty to the inch.

Such in outline is the description of the instrument, which, while scarcely adequate for very exact work, answered the purpose for which it was intended. It was constructed out of materials ready to hand or easily converted to serve the desired end. The method of using it is self suggestive. A cell was spun on an ordinary 3″ × 1″ slip and allowed to dry so as to become 'tacky' to the touch. A cover-glass, the thickness of which I had previously measured by means of a micrometer calliper, was then taken, and the index rod, removed from its bearings for the purpose, was cemented by marine glue to its exact centre, or as nearly as possible thereto. The cover-glass was then applied to the cell-wall and allowed to attach itself by its own weight or by very slight pressure. The slip had also been accurately gauged as to thickness, and after guaging the thickness of the same with the cell and cover-glass, by subtracting from the result the sum of the thickness of the cover-glass and slip, the remainder gave the initial thickness of the ring of cement whose shrinkage it was desired to measure. The slip was then put in place and rigidly secured by the screw at each end, and the index rod was put on its bearing. After bringing the index to zero the instrument was put under a bell-glass so arranged as to allow a free circulation of the atmosphere the of room and yet to protect it from interference or injury. (Unfortunately for me, however, it did not prevent the fall of a mounted deers-head and antlers, and the smashing into smithereens of the glass and instrument, and the serious injury of my Nachet stand, which occured some months afterwards). The following are the results attained:

Initial Depth of Cell Wall, =0.020''	Periods of Observation and Record.								Remarks.
	1 day.	2 days.	3 days.	10 days.	20 days.	30 days.	60 days.	90 days.	
Average Temperature, Fahr.....	68°	68°	70°	70°	70°	70°	72°	75°	These are the temperatures between observations and embrace parts of May and July.
Total Shrinkage up to each observation........	.001	.002	.0025	.0040	.0052	.0060	.0065	.0070	
Percentage of Shrinkage....	5	10	12½	20	26	30	32½	35	Showing total diminution of 35 per cent. in 90 days.

Owing to the unfortunate accident above referred to, a second series of measurments was interrupted in the sixth week, and I have been so closely occupied since that time that I have had no opportunity to reconstruct the apparatus. So far as it went, the second series absolutely confirmed the first in every particular, except that the weather being hotter and dryer, evaporation progressed much more rapidly.

The cement used in this series of observations contained originally about 40 per cent. of volatile matter, but had, very propably, lost a small portion of this by evaporation from the container before the series commenced. When the slide was taken off the instrument and the cell cut into, while the cement was not pulverulent when scraped, it was quite hard and dry, and would in all probability have shrunken but a very slight trifle more, if at all, under a prolongation of the tests.

Two things must be remembered here in taking the time and rate of shrinkage into consideration, viz: that had the cell been filled with glycerine the rate of evaporation, and consequently shrinkage, would have been much slower, and, on the contrary, had the cover-glass not been applied and the ring left open to the atmosphere all around it, this rate would have been more rapid.

We may therefore say that a cell made of white zinc cement will season sufficiently for safe use in about 90 days where the temperature remains in the neighborhood of 70° Fahr.

NOTE.—The loss of the original manuscript of this paper—its removal from the Secretary's table at Cleveland by somebody who failed to return it, has forced the author to rewrite it amid other and absorbing duties. The table however is taken from the original note-book and is quite accurate.